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VARIABILITY IN SOILS AND ITS SIGNIFICANCE TO PAST AND FUTURE SOIL INVESTIGATIONS

I. A STATISTICAL STUDY OF NITRIFICATION IN SOIL

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It is very generally recognized that different soils vary widely as regards their physical, chemical, and biological nature. It has also been recognized among soil investigators, at least, that different samples of the same soil type taken from a comparatively limited area may show considerable variation among themselves if we apply quantitative measurements to the various constituents of the soil mass. In any small area of the size usually employed in field experiments, these variations seem to have been regarded as of such limited magnitude as to be worthy of but secondary consideration. In many instances, a single sample from such an area has been taken and the assumption made that it represented the entire soil mass of the depth to which it was taken. In other words, the soil has been considered as a constant to which no corrections need be applied. Most workers in the field of soils have been content with taking relatively few samples and regarding determinations made upon the composite of these samples as accurate within the limits of error of the experiment. That the variations between different samples taken from a small area may be of such magnitudes as to bring experimental data obtained with one or a limited number of samples into very serious question, or even to invalidate such data entirely, seems not to have been considered. It is the purpose of this paper to emphasize the importance of this phase of soil investigation as regards both past and future endeavors.

It is obviously impossible, within the limits of a single paper, to consider the variations which characterize all the constituents of any

given soil, so that the results reported at the present time relate only to one phase of the biochemistry of soils. But few attempts have been made by investigators to determine actually the magnitude of variability in those products of microörganic activity which are capable of quantitative measurement and in no instance has a mathematical interpretation been attempted with such measurements. There are but three references [1, 2, 3] in the literature, as far as the writer is aware, dealing with this phase of the biochemistry of soils, and none of them is extensive enough to be of any value as statistical studies of variability. A number of papers[4] have appeared dealing with the variation in the weight of the crop produced over different parts of an apparently uniform field. Such variations reflect the variability of the soil, serving simply as a substratum for the growth of plants, but it is evident that the variations between such measurements as those given do not depend upon the soil as the only variable factor. Any attempt to correlate the crop produced on any given soil with the chemical composition of that soil, for instance, must necessarily take into account variations in both crop and soil. In fact, it appears to the writer that any correlation which may exist between the properties of any given soil and its crop-producing power can only be worked out by the statistical interpretation of data obtained as recorded below, together with the data secured in a similar manner for the crops produced on that soil. The problem of variability in the soil itself is worthy of careful experimental study, both from the standpoint of soil investigations in themselves and from that of their possible bearing upon the problems of variability in field experiments with crops. The results of such a study, as regards nitrate production, are presented below.

Methods

The field selected was one on the University Farm at Davis. For the three years preceding 1917, corn, Sudan grass, and grain sorghum had been grown in the order named. In 1917, the field was allowed to lie fallow and at the time the samples were taken (Oct. 20), was free of vegetation of any kind. No rain had fallen since April so that the surface soil was practically air-dry, the subsoil, however, being quite moist. The soil is classified as a silty clay loam. The particular area chosen was apparently as uniform as one could well find, being level, of uniform texture and color, and free from small local depressions of any kind.

The samples were taken at the locations as shown in figure 1, the diameter of the plot being one hundred feet. Each number in the following table represents two samples, soil and subsoil, the latter term being used for convenience in expression rather than to represent any sharp line of demarcation between the two samples taken at any given

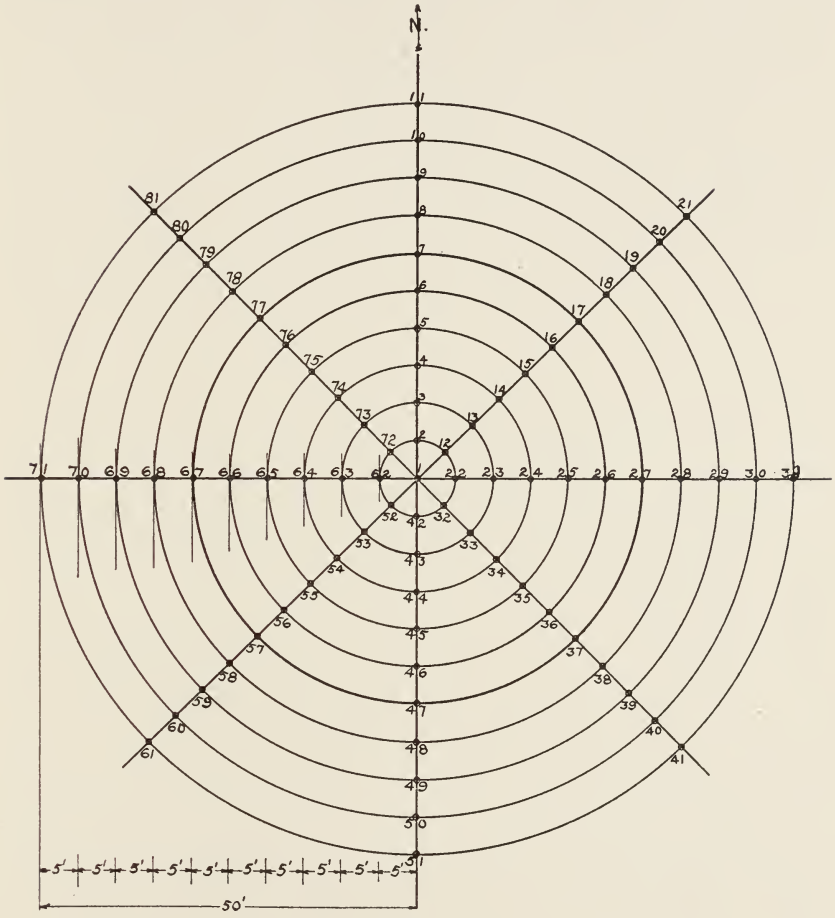

Fig. 1

place. All the surface samples were taken with a trowel to a depth of six inches, an area having a diameter of approximately six inches being included in each sample. The subsoil samples were taken with a three-inch auger to a depth of twenty-four inches and, therefore, represent the section from six to twenty-four inches in depth. All the samples were placed in sterile soil bags as soon as taken, the total amount of soil in each sample being about twice that necessary for the laboratory

determinations. Not more than twenty-four hours elapsed between the time the first sample was taken and the time all the samples were at the express office ready for shipment to Berkeley, where they arrived forty-eight hours later.

At the laboratory, the samples were allowed to air-dry for six days in the original canvas bags, this time for drying being necessary because of the moisture present in the subsoil samples. At the expiration of the six-day period all of the samples were sieved through a two-millimeter sieve and four one hundred gram portions of each sample weighed into tumblers. One tumbler from each sample was reserved for the determination of residual nitrate; to a second no nitrogen compounds were added, and to the others two-tenths of a gram of ammonium sulfate and one gram of dried blood, respectively, these amounts having been most frequently used in nitrification experiments in this laboratory. All the tumblers were brought to an optimum moisture content by the addition of twenty cubic centimeters of sterile distilled water and placed in the incubator at 28° C for twenty-eight days. During the incubation period, the water lost was replaced at weekly intervals. At the expiration of that period, the soil was dried at a temperature of 100° C and the nitrates determined colorimetrically by the phenoldisulfonic acid method as modified by Lipman and Sharp.[5] All results are reported as milligrams of nitrate nitrogen in one hundred grams of soil.

It was not deemed necessary to make duplicate determinations on all the samples, since from previous results secured in this laboratory, the variation between duplicates, as regards nitrification studies, is small and well within the limits of the error made in the readings, which were never recorded in this study further than to one-tenth of a milligram of nitrate nitrogen. Aside from this fact, it is doubtful if duplicate determinations are of value in experiments of this kind in which the variation between the samples was found to be so large.

The amount of nitrate nitrogen produced was chosen as the criterion of variability because of the generally accepted idea that nitrate nitrogen is more directly available to plants than other nitrogen combinations and for that reason more significance may rightly be attached to the amount of nitrate nitrogen found or produced in any given soil. Then, too, small amounts of nitrate may be determined rapidly, with a very fair degree of accuracy, and moreover, are less subject to fluctuations in the duplicate determination as discussed above. The absolute accuracy of the nitrate determination by the phenoldisulfonic

acid method is not of moment in this connection, because neither very low nor very high amounts of nitrate were ever determined and all of the determinations are directly comparable one with the other, since exactly the same procedure was followed in every case.

It must be emphasized that no attempt has been made to segregate the causes of variation and the results as given are the summation of all the factors which are of importance in causing differences between samples. All the work was done in a careful manner with due attention to detail, no new or modified procedure being attempted. The results of this study, therefore, are intended to serve as a basis for interpreting the mass of data which has already been obtained by soil biologists and to emphasize the extreme importance of applying statistical methods to results secured in the future before their value as contributions to science or practice can be recognized.

Calculations[6]

The amounts of nitrate found are reported in tables 1 to 4, following, the individual determinations always being given. The mathematical treatment will be discussed briefly from the data given in table 1, the discussion being applicable to all the tables as well.

The mean as given is obtained by dividing the sum of all the determinations by the number of determinations. This figure represents, therefore, a hypothetical composite sample of all the samples taken. The deviation from the mean of any determination is found by taking the difference between the mean and the individual result. The mean deviation of a series of determinations is an expression of the average amount any single observation taken at random is likely to differ from the mean of the series. This figure may be either plus or minus, but as the sign is of no importance as regards subsequent calculations, it is not recorded but may easily be found by inspection. The standard deviation (σ) is found, after the manner usual in statistical investigations, by squaring the deviation of each determination from the mean, taking the sum of the squares thus found, dividing this figure by the total number of determinations made and taking the square root of the quotient. The percentage ratio of the standard deviation to the mean expresses the coefficient of variability (*C.V.*) for a given series of determinations. It is an expression of the percentage deviation on either side of the mean, within which approximately two-thirds of the determinations may be expected to lie. The

coefficient of variability of the amount of nitrate as found in the field soil is high, no less than 25.9 ± 2.1 per cent in the surface six inches and 51.4 ± 3.3 per cent in the vertical section from six to twenty-four inches. The range, therefore, within which two-thirds of the determinations may be expected to fall is from 2.0 to 3.4 milligrams in the surface soil and from 0.3 to 1.1 milligram in the subsoil. The extreme range is, of course, much greater than this, but the bulk of the determinations fall within the limits given.

A single determination, or the mean of a series of determinations can never be an absolute value and hence before any large degree of confidence can be placed in any experimental result, its degree of reliability must be known. The reliability of any determination is expressed by the probable error (E) of the determination. This figure is of such a magnitude that the probability of making an error greater than it is equal to the probability of making an error less than it, both probabilities being one-half.

The probable error (E_s) of a single determination is calculated by the formula

$$E_s = \pm .6745 \times \frac{\sigma}{\sqrt{n}}$$

where σ is the standard deviation, as given above, and n the number of determinations. Since with a single determination the $\sqrt{n}$ is equal to 1

$$E_s = \pm .6745 \times \sigma.$$

or in other words, the probable error of a single variant is equal to approximately two-thirds of the standard deviation of the series in which it lies. The probable error of the mean (E_M), is given by the formula

$$E_M = \pm \frac{Es}{\sqrt{n}} = \frac{.6745 \times \sigma}{\sqrt{n}}$$

E_M will vary as the square root of the number of determinations and thus decreases but slowly as we increase the number of determinations. The probable error ($E\sigma$) of the standard deviation is calculated from the formula

$$E\sigma = \pm .6745 \frac{\sigma}{\sqrt{2n}}$$

and for the coefficient of variability:

$$Ec = .6745 \frac{C.V.}{\sqrt{2n}} \left[1 + 2\left(\frac{c}{100}\right)^2\right]^{1/2}$$

when $C.V.$ is greater than ten per cent as is the case with the results recorded below.

TABLE 1

RESIDUAL NITRATE IN SOIL AS SAMPLED

No.	1″–6″ Nitrate nitrogen Mgs.	1″–6″ Deviation from mean ± Mgs.	6″–24″ Nitrate nitrogen Mgs.	6″–24″ Deviation from mean ± Mgs.	No.	1″–6″ Nitrate nitrogen Mgs.	1″–6″ Deviation from mean ± Mgs.	6″–24″ Nitrate nitrogen Mgs.	6″–24″ Deviation from mean ± Mgs.
1	2.5	0.2	1.0	0.3	42	3.0	0.3	0.6	0.1
2	2.8	0.1	1.1	0.4	43	3.4	0.7	0.7	0.0
3	3.0	0.3	1.0	0.3	44	1.8	0.9	0.5	0.2
4	2.5	0.2	1.2	0.5	45	2.5	0.2	0.6	0.1
5	3.8	1.1	1.9	1.2	46	2.1	0.6	0.5	0.2
6	2.7	0.0	1.5	0.8	47	4.0	1.3	0.8	0.1
7	3.3	0.6	1.5	0.8	48	3.1	0.4	0.6	0.1
8	3.2	0.5	1.2	0.5	49	4.4	1.7	0.4	0.3
9	2.5	0.2	1.2	0.5	50	3.5	0.8	0.5	0.2
10	2.9	0.2	1.4	0.7	51	2.3	0.4	0.7	0.0
11	3.0	0.3	1.5	0.8	52	3.1	0.4	0.6	0.1
12	2.7	0.0	0.8	0.1	53	2.3	0.4	0.8	0.1
13	1.9	0.8	1.2	0.5	54	2.3	0.4	0.6	0.1
14	3.7	1.0	1.1	0.4	55	1.4	1.3	0.8	0.1
15	3.8	1.1	0.8	0.1	56	2.5	0.2	0.5	0.2
16	3.0	0.3	1.0	0.3	57	1.8	0.9	0.5	0.2
17	2.0	0.7	0.8	0.1	58	1.7	1.0	0.4	0.3
18	2.2	0.5	1.0	0.3	59	1.3	0.4	0.5	0.2
19	2.9	0.2	1.0	0.3	60	2.0	0.7	0.4	0.3
20	3.5	0.8	0.8	0.1	61	1.5	1.2	0.4	0.3
21	3.0	0.3	1.0	0.3	62	4.0	1.3	0.4	0.3
22	4.5	1.8	0.8	0.1	63	3.0	0.3	0.3	0.4
23	3.5	0.8	0.9	0.2	64	3.0	0.3	0.5	0.2
24	3.6	0.9	0.5	0.2	65	2.0	0.7	0.4	0.3
25	1.8	0.9	0.6	0.1	66	2.0	0.7	0.5	0.2
26	3.4	0.7	0.8	0.1	67	3.0	0.3	0.6	0.1
27	3.0	0.3	2.2	1.5	68	4.3	1.6	1.2	0.5
28	1.5	0.8	0.8	0.1	69	3.2	0.5	0.4	0.3
29	2.3	0.4	0.6	0.1	70	2.6	0.1	0.5	0.2
30	2.6	0.1	0.5	0.2	71	3.5	0.8	0.4	0.3
31	1.3	1.4	0.5	0.2	72	2.7	0.0	0.3	0.4
32	3.1	0.4	0.9	0.2	73	2.4	0.3	0.4	0.3
33	2.6	0.1	0.4	0.3	74	2.0	0.7	0.4	0.3
34	2.8	0.1	0.3	0.4	75	2.1	0.6	0.4	0.3
35	1.8	0.9	0.3	0.4	76	2.6	0.1	0.6	0.1
36	1.3	1.4	0.4	0.3	77	1.5	1.2	0.6	0.1
37	1.0	1.7	0.5	0.2	78	2.6	0.1	0.6	0.1
38	4.0	1.3	0.9	0.2	79	2.4	0.3	0.9	0.2
39	3.0	0.3	0.6	0.1	80	2.2	0.5	1.2	0.5
40	3.6	0.9	0.4	0.3	81	2.0	0.7	0.8	0.1
41	3.8	1.1	0.6	0.1					
					Mean	2.70±.05	0.5	0.70±.03	0.3
					σ	= .70±.04		σ = .36±.02	
					C.V.	= 25.9±2.1%		= 51.4±3.3%	
					E_M	= 1.8%		= 4.3%	

TABLE 2

NITRATE PRODUCED FROM THE SOIL'S OWN NITROGEN AFTER TWENTY-EIGHT DAYS' INCUBATION—INCUBATED BLANKS

	1″–6″		6″–24″			1″–6″		6″–24″	
No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.
1	4.3	0.9	1.2	0.2	42	4.5	1.1	1.2	0.2
2	2.4	1.0	1.0	0.4	43	3.8	0.4	1.4	0.0
3	2.2	1.2	1.0	0.4	44	3.0	0.4	1.2	0.2
4	1.6	1.8	0.8	0.6	45	4.0	0.6	1.0	0.4
5	2.7	0.7	1.1	0.3	46	3.0	0.4	1.2	0.2
6	4.0	0.6	1.2	0.2	47	3.8	0.4	1.3	0.1
7	1.8	1.6	1.8	0.4	48	3.3	0.1	1.2	0.2
8	3.0	1.4	1.0	0.4	49	5.2	1.8	1.2	0.2
9	4.8	1.4	2.2	0.8	50	4.8	1.4	1.4	0.0
10	3.0	0.4	3.0	1.6	51	4.0	0.6	2.0	0.6
11	4.2	0.8	2.1	0.7	52	3.5	0.1	1.6	0.2
12	4.5	1.1	1.5	0.1	53	3.1	0.3	0.8	0.6
13	3.1	0.3	1.4	0.0	54	4.7	1.3	1.5	0.1
14	4.6	1.2	1.5	0.1	55	3.2	0.2	0.7	0.7
15	4.2	0.8	1.1	0.3	56	4.1	0.7	1.2	0.2
16	4.2	0.8	1.6	0.2	57	1.2	1.2	2.0	0.6
17	2.4	1.0	1.5	0.1	58	3.4	0.0	1.0	0.4
18	2.8	0.6	1.1	0.3	59	2.0	1.4	1.0	0.4
19	1.8	1.6	3.0	1.6	60	3.0	0.4	2.2	0.8
20	4.8	1.4	2.2	0.8	61	1.0	2.4	0.5	0.9
21	4.8	1.4	1.4	0.0	62	1.5	1.9	0.6	0.8
22	3.5	0.1	1.5	0.1	63	1.1	2.3	0.7	0.7
23	3.7	0.3	1.4	0.0	64	1.0	2.4	1.7	0.3
24	3.0	0.4	1.2	0.2	65	3.1	0.3	1.3	0.1
25	4.2	0.8	1.7	0.3	66	5.0	1.6	1.8	0.4
26	3.8	0.4	1.8	0.4	67	3.6	0.2	1.5	0.1
27	3.1	0.3	1.6	0.2	68	2.1	1.3	2.0	0.6
28	2.8	0.6	1.4	0.0	69	4.4	1.0	1.1	0.3
29	4.8	1.4	1.5	0.1	70	3.1	0.3	1.2	0.2
30	3.5	0.1	1.5	0.1	71	5.5	2.1	1.1	0.3
31	2.8	0.6	0.4	0.0	72	3.6	0.2	1.4	0.0
32	3.2	0.2	2.0	0.6	73	3.8	0.4	1.0	0.4
33	5.2	1.6	1.8	0.2	74	3.8	0.4	0.3	1.1
34	3.3	0.1	1.2	0.2	75	5.4	2.0	1.2	0.2
35	4.5	1.1	1.1	0.3	76	3.8	0.4	1.2	0.2
36	2.8	0.6	1.4	0.0	77	4.6	1.2	1.6	0.2
37	3.0	0.4	3.7	2.3	78	1.5	1.9	1.5	0.1
38	1.7	1.7	1.1	0.3	79	5.0	1.6	1.8	0.4
39	3.8	0.4	1.2	0.2	80	3.1	0.3	1.9	0.5
40	4.0	0.6	1.7	0.3	81	3.5	0.1	1.5	0.4
41	4.1	0.7	1.0	0.4					
					Mean	3.40±.08	0.9	1.40±.03	0.4
					σ	= 1.06±.05		σ = .50±.02	
					C.V.	= 31.2±1.7%		= 35.7±2.1%	
					E_M	= 2.3%		= 2.1%	

TABLE 3

NITRATE PRODUCED FROM 0.2 GRAM OF AMMONIUM SULPHATE IN 100 GRAMS OF SOIL

	1″–6″		6″–24″			1″–6″		6″–24″	
No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.
1	6.6	1.6	1.8	1.0	42	2.1	2.9	6.3	3.5
2	4.3	0.7	3.7	0.9	43	6.2	1.2	3.0	0.2
3	8.0	3.0	2.9	0.1	44	5.2	0.2	1.0	1.8
4	6.0	1.0	3.3	0.5	45	2.3	2.7	1.2	1.6
5	4.2	0.8	3.3	0.5	46	1.8	3.2	1.8	1.0
6	6.0	1.0	2.5	0.3	47	2.5	2.5	1.5	1.3
7	5.6	0.6	3.2	0.4	48	2.5	2.5	1.2	1.6
8	5.0	0.0	4.3	1.5	49	2.0	3.0	1.2	1.6
9	5.8	0.8	4.0	1.2	50	2.3	2.7	2.2	0.6
10	5.3	0.3	3.8	1.0	51	4.8	0.2	2.3	0.5
11	4.0	1.0	1.1	1.7	52	5.5	0.5	1.7	1.1
12	5.5	0.5	3.0	0.2	53	5.4	0.4	1.7	1.1
13	5.0	0.0	2.8	0.0	54	6.5	1.5	2.8	0.0
14	4.6	0.4	2.8	0.0	55	2.3	2.7	3.2	0.4
15	5.0	0.0	3.1	0.3	56	5.2	0.2	1.2	1.4
16	6.5	1.5	3.3	0.5	57	2.2	2.6	1.5	1.3
17	4.6	0.4	5.7	1.9	58	3.6	1.4	3.2	0.4
18	5.0	0.0	4.0	1.2	59	2.2	2.6	5.0	2.2
19	6.0	1.0	4.0	1.2	60	3.5	1.5	6.0	3.2
20	5.0	0.0	2.4	0.4	61	4.2	0.8	3.5	0.7
21	5.4	0.4	2.8	0.0	62	7.2	2.2	2.0	0.8
22	6.0	1.0	3.4	0.6	63	7.3	2.3	2.4	0.4
23	6.5	1.5	3.2	0.4	64	3.7	1.3	4.1	1.3
24	4.8	0.2	2.0	0.8	65	5.7	0.7	0.2	0.0
25	5.3	0.3	2.7	0.1	66	3.8	1.2	2.5	0.3
26	5.5	0.5	5.4	2.6	67	4.9	0.1	3.0	0.2
27	4.4	0.6	4.8	2.0	68	4.8	0.2	4.0	1.2
28	7.5	2.5	4.7	1.9	69	6.8	1.8	1.0	1.8
29	7.6	2.6	2.8	0.0	70	5.8	0.8	1.3	1.5
30	6.2	1.2	1.9	0.9	71	4.0	1.0	1.0	1.8
31	4.2	0.8	2.6	0.2	72	1.8	3.2	4.0	1.2
32	5.0	0.0	3.2	0.4	73	5.3	0.3	2.3	0.5
33	6.0	1.0	3.0	0.2	74	5.8	0.8	3.8	1.0
34	4.5	0.5	2.2	0.6	75	8.2	3.2	1.5	1.3
35	4.8	0.2	1.6	1.2	76	5.5	0.5	2.5	0.3
36	4.8	0.2	1.5	1.3	77	6.2	1.2	3.1	1.3
37	4.0	1.0	2.6	0.2	78	6.0	1.0	3.0	1.2
38	5.4	0.4	2.5	0.3	79	6.3	1.3	2.6	0.2
39	5.2	0.2	2.8	0.0	80	6.0	1.0	3.5	0.7
40	6.6	1.6	2.0	0.8	81	6.0	1.0	3.2	0.4
41	6.4	1.4	2.0	0.8					
					Mean	5.00±.10	1.0	2.80±.08	0.9
					σ	= 1.50±.08		σ = 1.10±.07	
					C.V.	= 29.4±1.6%		= 40.7±2.4%	
					E_M	= 2.0%		= 3.4%	

TABLE 4

NITRATE PRODUCED FROM 0.2 GRAM OF BLOOD IN 100 GRAMS OF SOIL

No.	1″–6″ Nitrate nitrogen Mgs.	1″–6″ Deviation from mean ± Mgs.	6″–24″ Nitrate nitrogen Mgs.	6″–24″ Deviation from mean ± Mgs.	No.	1″–6″ Nitrate nitrogen Mgs.	1″–6″ Deviation from mean ± Mgs.	6″–24″ Nitrate nitrogen Mgs.	6″–24″ Deviation from mean ± Mgs.
1	27.0	6.0	11.0	0.3	42	7.0	14.0	20.0	9.5
2	20.0	1.0	10.0	0.7	43	18.0	3.0	10.0	0.5
3	13.0	8.0	14.0	3.3	44	18.0	3.0	13.0	2.3
4	30.0	9.0	15.0	4.3	45	21.0	0.0	12.0	1.3
5	18.0	3.0	25.0	14.3	46	13.0	8.0	14.0	3.3
6	40.0	19.0	25.0	14.3	47	18.0	3.0	19.0	8.3
7	20.0	1.0	18.0	7.3	48	20.0	1.0	12.0	1.3
8	14.0	7.0	16.0	5.3	49	21.0	0.0	18.0	7.3
9	20.0	1.0	10.0	0.7	50	20.0	1.0	17.0	6.3
10	22.0	1.0	16.0	5.3	51	24.0	3.0	9.0	1.7
11	18.0	3.0	14.0	3.3	52	21.0	0.0	10.0	0.7
12	22.0	1.0	12.0	1.3	53	27.0	6.0	12.0	1.3
13	23.0	2.0	13.0	2.3	54	32.0	11.0	22.0	11.3
14	22.0	1.0	5.5	5.2	55	32.0	11.0	7.0	3.7
15	16.0	5.0	5.6	5.1	56	20.0	1.0	18.0	7.3
16	21.0	0.0	7.0	3.7	57	27.0	6.0	10.0	0.7
17	16.0	5.0	8.0	2.7	58	27.0	6.0	4.3	6.4
18	20.0	1.0	5.0	5.7	59	14.0	7.0	3.0	7.7
19	21.0	0.0	5.5	5.2	60	30.0	9.0	20.0	9.5
20	13.0	8.0	7.7	3 0	61	22.0	1.0	4.3	6.4
21	24.0	3.0	5.0	5.7	62	23.0	2.0	10.0	0.7
22	15.0	6.0	10.0	0.7	63	24.0	3.0	9.0	1.7
23	22.0	1.0	14.0	3.5	64	14.0	7.0	4.8	5.9
24	24.0	3.0	8.0	2.7	65	29.0	8.0	10.0	0.7
25	19.0	2.0	8.0	2.7	66	21.0	0.0	10.0	0.7
26	20.0	1.0	10.0	0.7	67	28.0	7.0	6.5	4.2
27	21.0	0.0	20.0	9.5	68	23.0	2.0	1.7	0.0
28	21.0	0.0	12.0	1.3	69	19.0	2.0	5.5	5.2
29	20.0	1.0	4.4	6.3	70	16.0	5.0	3.7	7.0
30	11.0	10.0	5.9	4.8	71	17.0	4.0	2.7	8.0
31	20.0	1.0	7.4	3.3	72	27.0	6.0	4.0	6.7
32	17.0	4.0	14.0	3.3	73	30.0	9.0	5.3	5.4
33	10.0	11.0	13.0	2.3	74	28.0	7.0	24.0	13.3
34	20.0	1.0	5.0	5.7	75	25.0	4.0	10.0	0.7
35	15.0	6.0	2.4	8.3	76	22.0	1.0	12.0	1.5
36	12.0	9.0	8.5	2.2	77	28.0	7.0	4.2	6.5
37	19.0	2.0	11.0	0.3	78	24.0	3.0	9.0	1.7
38	16.0	5.0	14.0	2.3	79	22.0	1.0	12.0	1.3
39	14.0	7.0	8.0	2.7	80	17.0	4.0	7.2	3.5
40	13.0	8.0	5.7	5.0	81	30.0	9.0	21.0	10.3
41	27.0	6.0	11.0	0.3					
					Mean	21.10±0.4	4.0	10.70±0.4	4.5
					σ	= 5.70±0.30		σ = 5.30±.30	
					C.V.	= 27.1±1.5%		= 49.4±3.1%	
					E_M	= 1.9%		= 4.0%	

TABLE 5

Summary of Statistical Data

	Mean Milligrams	Extremes Milligrams	Standard deviation Milligrams	Coefficient of variability Per cent	Probable error of mean ± Per cent
Residual nitrate.					
Surface	2.70±.05	1.4–4.5	0.70±.04	25.9± 2.1	1.8
Subsoil	0.70±.03	0.3–1.2	0.36±.02	51.4± 3.3	4.3
Incubated blanks.					
Surface	3.40±.08	1.0–5.5	1.06±.05	31.2± 1.7	2.3
Subsoil	1.40±.08	0.1–2.2	0.50±.02	35.7± 2.1	2.1
$(NH_4)_2SO_4$.					
Surface	5.00±.10	1.8–8.2	1.50±.08	29.4± 1.6	2.0
Subsoil	2.80±.08	0.2–3.2	1.10±.07	40.7± 2.4	3.4
Blood.					
Surface	21.00±.40	7.0–40.0	5.70±.30	27.1± 1.5	1.9
Subsoil	10.70±.40	1.7–25.0	5.30±.30	49.4± 3.1	4.0

Unless otherwise stated, the probable error of any value has been used directly as found in the discussion which follows so that there is simply an even chance that the results so treated fall within, or without, the limits of their respective probable errors. The effect of multiplying the probable error by two, three, or any higher number, may be found by referring to the reference cited above.

Discussion of Experimental Results

To express the results in concise form, table 5 has been inserted to summarize briefly the data of the preceding tables. In the first column the means of the various series are given with their respective probable errors. In the case of residual nitrate, 2.7 milligrams in one hundred grams of soil represent .0027 per cent of the soil or approximately 54 pounds of nitrate nitrogen per acre, considering only the upper six inches of the soil mass. The probable error of the determination amounts to .00005 per cent of the soil so that it is an even chance that the mean of the eighty-one determinations is correct within one pound per acre. In the subsoil, the mean of 14 pounds per acre, is within 0.6 pounds of the correct figure, on the same basis. The extreme range amounts to 62 pounds in the surface soil and 18 pounds per acre in the subsoil, both figures being of greater magnitude than the means of the two series. It is not possible to translate the other figures into a practical pounds per acre basis, since they represent laboratory treatments.

The extremes recorded in column two are simply the extreme determinations found in any one series. The extreme range may be determined by taking the difference between these two figures. The greatest extremes are shown by the samples to which dried blood was added, with the ammonium sulfate samples a close second. The results for any one series emphasize the very large difference found between a large number of samples treated as uniformly as possible.

It will be noted that the coefficients of variability as regards the surface samples with their various treatments differ but little among themselves, this difference amounting to only 5.3 per cent. The difference is greater with the four series in which the subsoil was used, being 15.7 per cent or nearly three times that of the surface samples. The point is again emphasized here that we are dealing with the summations of all the errors to which the samples are subject, both field and laboratory. One source of error has been allowed for, which is of a purely mechanical nature, namely, that of making the readings with the colorimeter. This error will be considered in the following section, and the coefficients of variability discussed at greater length there.

The percentage ratios given as the probable error of the mean place all the results on a comparable basis as regards the error to which the various means are subject. Attention is called to the fact that these figures are of similar magnitudes in the four series of surface samples while showing much larger differences in the subsoil samples with their various treatments.

It will be noted from tables 1, 2, and 3 that in a number of samples there was less actual nitrate nitrogen found in the subsoil samples after incubation without the addition of anything but water, or with the addition of 0.2 gram of ammonium sulphate, than was present in the soil of corresponding samples as they came from the field. This result was not anticipated and no explanation to account for this loss of nitrate nitrogen is offered at the present time. It is, however, regarded as of biochemical interest largely and not of importance as regards the variation between samples at the time the determinations were actually made.

Error of the Determinations Due to the Colorimeter

Reference has already been made to the absolute accuracy of the nitrate determination and its bearing upon the results reported in the present paper. Aside from the absolute accuracy of the determina-

tions, it is desired to consider briefly the error in making the readings on the colorimeter due to the inability of the eye to detect small changes in the depth of color between the standard employed and the unknown solutions. All workers in soil chemistry are familiar with the Kenicott-Sargent colorimeter, so that the instrument itself needs no description here. To check the readings on the unknown solutions, a solution was prepared of the average strength of the residual nitrate determined in the first series reported. Sixteen equal volumes of this solution were taken and treated exactly as the soil extracts were treated. The average amount of nitrate nitrogen found in the sixteen portions was the same as that for the series referred to above, namely, 2.7 milligrams. The actual determinations, together with the calculated statistical constants, are reported in table 6. The probable error of the mean of sixteen samples, together with the error to which both larger and smaller numbers of samples are subject, is given in table 7. The calculations have been made by the use of the formulae already given. It is evident that the calculation of the probable error from sixteen, instead of a larger number of samples, makes it less reliable than if a larger number had been used, but since the error from this source is relatively small as compared to the error due to field sampling, it is deemed of sufficient accuracy for the purpose in hand. This error will be referred to as the laboratory error to distinguish it from the error due to sampling.

TABLE 6

COEFFICIENT OF VARIABILITY AND PROBABLE ERROR OF COLORIMETER READINGS

No.	Nitrate nitrogen Milligrams	Deviation from mean ± Milligrams	No.	Nitrate nitrogen Milligrams	Deviation from mean ± Milligrams
1	2.5	0.2	9	2.6	0.1
2	2.8	0.1	10	2.8	0.1
3	2.8	0.1	11	3.0	0.3
4	2.9	0.2	12	2.6	0.1
5	2.5	0.2	13	2.7	0.0
6	2.6	0.1	14	2.7	0.0
7	2.8	0.1	15	2.6	0.1
8	2.6	0.1	16	2.7	0.0
			Mean	2.7±.02	0.1
			σ	= .13±.01	
			C.V.	= 4.8±.5%	
			E_M	= 0.8%	

It will be noted that the probable error of a single nitrate determination is 2.7 ± .09 milligrams or 3.2 per cent of the amount determined. The error for a single determination, expressed on the field samples as a percentage, is 17.4 or about 5.1 times greater than the laboratory error, even after making allowance for this error. With sixteen determinations, the probable error becomes 2.7 ± .022 milligrams or 0.8 per cent. Further, with eighty-one samples, the probable error is only 2.7 ± .009 milligrams or 0.3 per cent.

In allowing for this laboratory error in the various series reported, it is evident that it becomes relatively larger the smaller the amount of nitrate determined within the limits of the amounts found in the present study. In other words, the probable error will remain the same while the amount of nitrate determined decreases, so that the probable error will form a larger percentage of the determination. On the other hand, the probable error becomes relatively smaller as we increase the actual amount of nitrate nitrogen again within the limits of the amounts reported here. The same is true for the coefficient of variability. This increased ratio is brought out in table 8, which is simply a reconstruction of table 5, after making allowance for the laboratory error.

The coefficient of variability computed from the standard deviation (.13 ± .01 mg.) and the average amount, of nitrate nitrogen reported for the various series is given in column one. This figure is the largest for the subsoil samples in the field and the smallest for the surface samples treated with blood, the mean of the eighty-one readings being 0.7 and 21.0 milligrams, respectively, in the two cases. In the second column, the corrected coefficients of variability for the field samples are given, these being the difference between coefficients of variability before the laboratory error was allowed for and the various coefficients of variability given in column one. It will be noted that the same

TABLE 7

SHOWING THE DECREASE OF LABORATORY ERROR AS NUMBER OF DETERMINATIONS INCREASE

Number of samples	Probable error of mean Milligrams	Probable error of mean Per cent	Number of samples	Probable error of mean Milligrams	Probable error of mean Per cent
1	2.700±.087	3.2	36	.014	0.5
4	.043	1.6	49	.012	0.4
9	.039	1.1	64	.011	0.4
16	.022	0.8	81	.009	0.3
25	.017	0.7			

qualitative relations hold as between the various treatments in every case except the incubated subsoil samples which are somewhat less variable than the surface samples. With the other three series, the subsoil samples still show a much higher coefficient of variability than the surface samples, as do the samples treated with fertilizers in the laboratory in contrast with the untreated field samples. The percentage probable errors or laboratory errors are shown in column three, while the corrected figures for the field samples are given in column four.

Referring again for a moment to table 6, it is evident that the laboratory error increases as the square root of the number of determinations made, so that the mean of any number of readings on the colorimeter becomes less reliable, the fewer the number, exactly as the mean of a fewer number of samples is less accurate than the mean of a larger number. In other words, the curves of the errors of the various determinations are parallel, as will be seen by reference to figure 2. This fact must be kept in mind in considering the results given in the following section.

Results of Random Samplings

It is of very direct interest to consider for a moment the accuracy of the mean of a limited number of samples taken at random. Ten surface samples are included in the first group (table 9) and sixteen in the second (table 10), since about these numbers of samples have

TABLE 8

Summary of Statistical Data after Making Allowance for the Laboratory Error

	Coefficient of variability (laboratory error) Per cent	Corrected coefficient of variability Per cent	Laboratory error Per cent	Corrected probable error of mean Per cent
Residual nitrate.				
Surface	4.8± 0.2	21.1± 2.1	0.3	1.5
Subsoil	18.5± 1.0	32.9± 3.4	1.2	3.1
Incubated blanks.				
Surface	3.8± 0.2	27.4± 1.7	0.3	2.0
Subsoil	9.2± 0.5	25.5± 2.1	0.7	1.4
Ammonium sulfate.				
Surface	2.6± 0.1	26.8± 1.6	0.2	1.8
Subsoil	4.6± 0.2	36.1± 2.4	0.4	3.0
Blood.				
Surface	0.6± 0.03	26.5± 1.5	0.05	1.9
Subsoil	1.2± 0.10	48.2± 3.1	0.09	3.9

frequently been used in making up a composite sample. It is assumed for the time being that the amount of nitrate actually found in a composite sample is that expressed by the mean of any given number of samples. All the calculations have been made upon the samples reported in the tables below just as if these were the only samples taken from the area, as would be done if such a number of samples were used in an independent investigation. In this case, however, we have a much larger number of samples to check the accuracy of the results obtained with the fewer number. Table 9 gives the amounts of nitrate found in ten surface samples, numbered from forty-two to

TABLE 9

VARIABILITY OF TEN SURFACE SAMPLES TAKEN AT RANDOM

	Residual nitrate		Incubated blanks		$(NH_4)_2SO_4$		Blood	
No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.
42	3.0	0	4.5	0.6	2.1	1.0	7.0	10.0
43	3.4	0.4	3.8	0.1	6.2	3.1	18.0	1.0
44	1.8	1.2	3.0	0.9	5.2	2.1	18.0	1.0
45	2.5	0.5	4.0	0.1	2.3	0.8	21.0	3.0
46	2.1	0.9	3.0	0.9	1.8	1.3	13.0	4.0
47	4.0	1.0	3.8	0.1	2.5	0.6	18.0	1.0
48	3.1	0.1	3.3	0.6	2.5	0.6	20.0	3.0
49	4.4	1.4	5.2	1.3	2.0	1.1	21.0	4.0
50	3.5	0.5	4.8	0.9	2.3	0.8	20.0	3.0
51	2.3	0.7	4.0	0.1	4.8	0.7	24.0	7.0
Mean	3.00±1.7	0.6	3.90±.15	0.6	3.10±.31	1.3	17.00±.89	3.7
σ	0.80±0.12		0.70±.10		1.50±.22		4.70±.63	
C.V.	26.7±4.3%		17.9±2.7%		48.4±3.9%		24.7±3.8%	
E_M	5.7%		3.8%		10.0%		5.2%	

fifty-one, inclusive. It will be noted that these samples are taken in a straight line (see fig. 1), while the sixteen samples are taken indiscriminately over the area. Considering the residual nitrate alone, it is seen that the mean of the ten samples is 0.30 milligrams above the mean of the total number of samples. Further, the probable error of the mean is increased from ± .05 milligram to ± .17 milligram or, in terms of the probable error, the chances are about 3 to 1 that the difference between the two results is a significant one.* The coefficients of variability are very nearly the same in the two instances.

* The probable error of the differences between two results is calculated from the formula:

$$\text{Probable error of difference} = \sqrt{E_1^2 + E_2^2}$$

In the incubated samples, the probable error of the difference between the samples is 0.50 ± .17 milligrams, for the samples to which ammonium sulfate was added 1.90 ± .31 milligrams, and for dried blood 4.00 ± .97 milligrams. It is worthy of note that the probable error of the differences between eighty-one samples, to which ammonium sulfate was added, and the ten samples given above is no less than 6 to 1.† The coefficient of variability of the ten samples is greatly increased.

TABLE 10

VARIABILITY OF SIXTEEN SURFACE SAMPLES TAKEN AT RANDOM

	Residual nitrate		Incubated blanks		$(NH_4)_2SO_4$		Blood	
No.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.	Nitrate nitrogen Mgs.	Deviation from mean ± Mgs.
1	2.5	2.0	4.3	1.0	6.6	2.2	27.0	8.0
3	3.0	0.5	2.2	1.1	8.0	3.6	13.0	6.0
7	3.3	0.8	1.8	1.5	6.6	2.2	20.0	1.0
9	2.5	0.0	4.8	1.5	5.8	1.4	20.0	1.0
30	2.6	0.1	3.5	0.2	6.2	1.8	11.0	8.0
34	2.8	0.3	3.3	0.0	4.5	0.1	20.0	1.0
37	1.0	1.5	3.0	0.3	4.0	0.4	19.0	0.0
40	3.6	1.1	4.0	0.7	6.6	2.2	13.0	6.0
42	3.0	0.5	4.5	1.2	2.1	2.3	7.0	12.0
46	2.1	0.4	3.0	0.3	1.8	1.6	13.0	6.0
49	4.4	1.9	5.2	1.9	2.0	2.4	21.0	2.0
53	2.3	0.2	3.1	0.2	6.4	2.0	27.0	8.0
57	1.8	0.7	1.2	2.1	2.2	2.2	27.0	8.0
59	1.3	1.2	2.0	1.3	2.2	2.2	14.0	5.0
60	2.0	0.5	3.0	0.3	3.5	0.9	30.0	11.0
72	2.7	0.2	3.6	0.3	1.8	2.6	27.0	8.0
Mean	2.50±.14	0.6	3.30±.17	0.9	4.40±.35	1.9	19.00±1.1	6.0
σ	0.80±.10		1.00±.12		2.10±.26		6.70±.08	
C.V.	34.4±4.5%		30.3±3.9%		47.7±6.4%		35.2±4.6%	
E_M	5.6%		5.2%		7.9%		5.8%	

being 48.4 ± 3.9 per cent. On the other hand, it happens that the incubated blanks vary even less than the eighty-one samples, the coefficients of variability being 17.9 ± 2.7 per cent and 31.2 ± 1.7 per cent respectively. It is seen from these results that very wide variations may be found when only ten samples are used in making a composite sample and that that number of determinations is by no means enough to be an accurate measure of the actual nitrate nitrogen content when the variations are of the magnitudes of those noted above.

Turning for a moment to the results given in table 10, we find that

† For table of odds to aid in estimating the significance of differences between two results, see Batchelor and Reed, Jour. Agr. Res., vol. 12, pp. 265–266, 1918.

the probable error of the difference between the mean of the sixteen samples taken purely at random, and the entire number of determinations made, to be .20 ± .15 milligrams, with a coefficient variability of 34.4 ± 4.5 per cent as contrasted with 25.9 ± 2.1 per cent for the total number. The difference between the number of samples considered here and the total number is not significant with the incubated blanks, but with the ammonium sulfate samples the probable error of the difference between the means is 0.66 ± .33, and with dried blood 2.0 ± 1.1. The chances are somewhat less than 2 to 1 that the differences between the sixteen samples here considered and the total eighty-

TABLE 11

EFFECT OF DISTANCE UPON THE VARIABILITY IN SAMPLING—RESIDUAL NITRATE

Five-foot radius			Twenty-five foot radius			Fifty-foot radius		
No.	Nitrate nitrogen Milligrams	Deviation from mean Milligrams	No.	Nitrate nitrogen Milligrams	Deviation from mean Milligrams	No.	Nitrate nitrogen Milligrams	Deviation from mean Milligrams
2	2.8	0.4	6	2.7	0.3	11	3.0	0.5
12	2.7	0.5	16	3.0	0.6	21	3.0	0.5
22	4.5	1.3	26	3.4	1.0	31	1.3	1.2
32	3.1	0.1	36	1.3	1.1	41	3.8	1.3
42	3.0	0.2	46	2.1	0.3	51	2.3	0.2
52	3.1	0.1	56	2.5	0.1	61	1.5	1.0
62	4.0	0.8	66	2.0	0.4	71	3.5	1.0
72	2.7	0.5	76	2.6	0.2	81	2.0	0.5
Mean	3.20±.14	0.5		2.40±.14	0.4		2.50±.19	0.6
σ	0.60±.10			0.60±.10			0.80±.13	
C.V.	18.7±3.3%			25.0±4.5%			32.0±5.8%	
E_M	4.4%			5.8%			7.6%	

one samples are significant. It is evident that calculations made from the mean of sixteen samples are of a higher degree of accuracy than when only ten samples are used, but the number is still too few to give results of a high order of reliability, and could by no means be taken as truly representative of the entire area under consideration.

Other numbers and other groupings may be selected and the magnitude of their means and their accompanying probable errors and coefficients of variability computed from the data reported in tables 1 to 4.

EFFECT OF DISTANCE UPON VARIABILITY

To determine whether or not the distance apart the samples were taken is a factor of importance in sampling, the arrangement given in table 11 has been made. The results shown in the table are for residual

nitrate in the surface samples only. The first group of determinations, from numbers two to seventy-two, inclusive, by intervals of ten, were recorded from samples lying within a radius of five feet of number one. The mean of the eight readings is 3.20 ± .14 milligrams or 0.5 ± .15 milligrams above the mean for the whole number of samples. The coefficient of variability is, however, relatively low.

The second group of determinations from numbers six to seventy-six, varying as those of the group above, are of the samples on the circle with a radius of twenty-five feet from the center. The mean of this group of eight determinations is 2.40 ± .14 milligrams or 0.3 ± .15 below that of the established mean as already given. The coefficient of variability in this case is nearly the same as for the total of eighty-one samples, however.

The last group of determinations represents the nitrate found in the samples taken as the fifty-foot radius. The mean of the eight determinations is 2.50 ± .20 milligrams; 0.70 ± .24 milligrams and .10 ± .24 milligrams. In two cases, the differences are significant; in the third, the probable error is greater than the difference and holds between the most widely separated samples taken on the twenty-five and fifty-foot radii. Even though only eight samples are considered in any one group, the conclusion seems justified that the distances apart samples are taken is of little importance, except in so far as their distribution be uniform over the area to be sampled. A small area of an apparently uniform field may lead to very erroneous results, as evidenced by the high figures obtained for the mean of the determinations made upon the samples on the five-foot radius. It is, of course, taken for granted that we are dealing in every instance with a field suitable for experimental work and hence not marked by changes in the soil apparent to the eye.

Estimate of the Number of Samples Required for any Given Degree of Accuracy[7]

It is very desirable to know just how many samples it is necessary to use to secure the degree of accuracy deemed desirable for the work in hand. By the use of the probable error and the standard deviation found from any representative number of samples, an estimate of the number of samples which will give a lower or higher degree of accuracy than the number used may be computed. For example, in the case of

the residual nitrate determinations, we have seen that the mean of the eighty-one samples amounted to 2.70 milligrams, with a probable error of $\pm .05$ milligrams and the standard deviation of the series 0.70 gram. As already stated, the probable error of the mean is expressed as

$$E_M = \frac{.6745 \times \sigma}{\sqrt{n}}$$

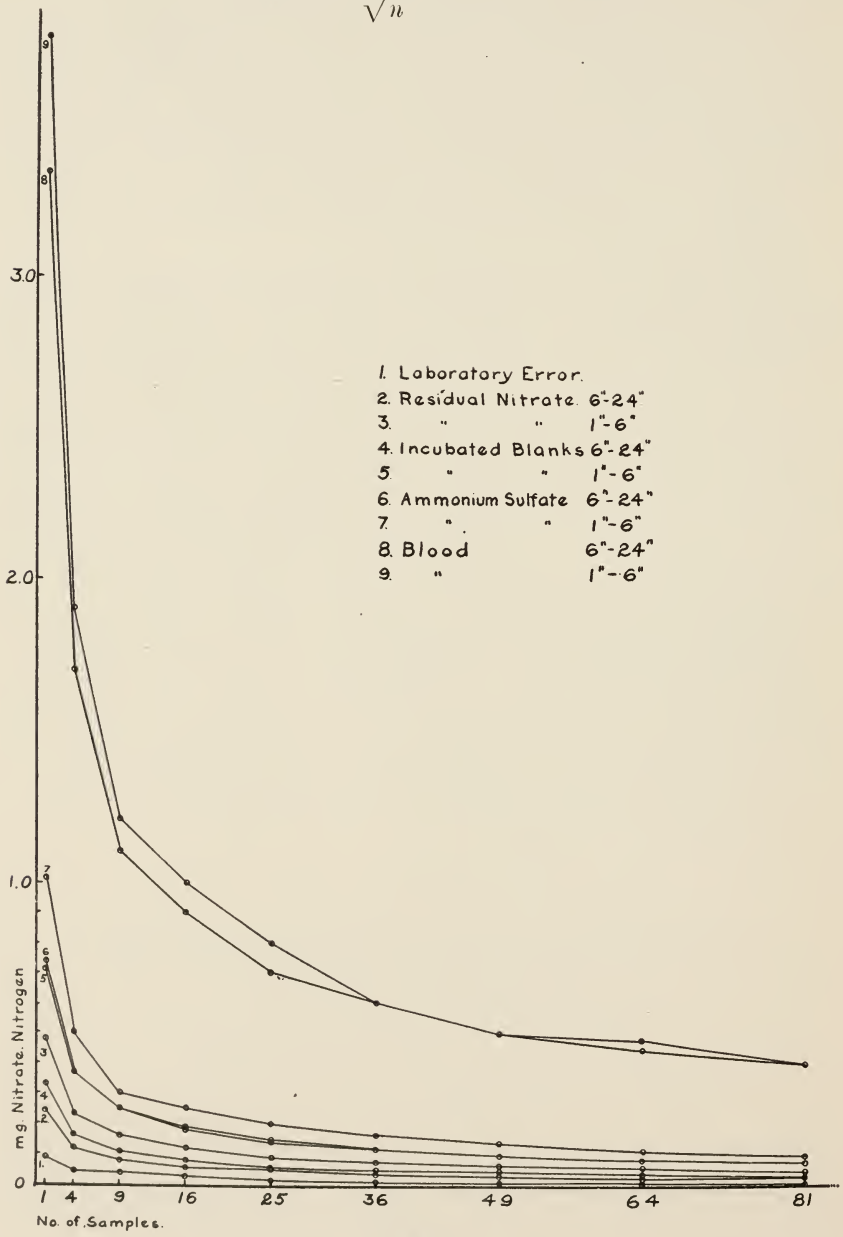

Fig. 2

and since the standard deviation in this instance is 0.7 gram, hence

$$E_M = \frac{.6745 \times 0.70}{\sqrt{n}}$$

We can make E_M of any dimension desired and since our method of determining nitrates allows of direct determinations only to 0.1 milligram, we will use this number for the probable error of the mean of the desired number of determinations so that

$$\pm 0.10 = \frac{.6745 \times 0.70}{\sqrt{n}}$$

from which $n = 22$. It must be remembered, however, that the laboratory error increases as we decrease the number of determinations in the same manner as the probable error of our sampling, so that this increased error must be taken into account in estimating the number of samples necessary to ensure any desired degree of accuracy. Referring to table 7, we find that the probable error of making the readings on the colorimeter for twenty-five samples is .017 milligram or very nearly .018 milligram for twenty-two samples, so that for a probable error of 0.1 milligram we have

$$\pm 0.1 = \frac{.6745 \times 0.13}{\sqrt{n}}$$

and $n = 1$. Thus twenty-three samples are sufficient so that the probable error in the mean is 0.10 milligram. The taking into account of the laboratory error involves an extra calculation, which, for all practical purposes, may be avoided by the use of a table, such as shown by table 12 (represented graphically in figure 2), which has been calculated from the data given in tables 1, 2, 3, and 4, after the manner already outlined. It will be noted that this table is of limited range and accounts for numbers of samples less than eighty-one, but may be extended for a range greater than the one given if desired. The approximate number of samples may be readily found after the following manner. It is desired to determine the number of ammonium sulfate samples necessary to be taken to ensure the same degree of accuracy as for twenty-three residual nitrate samples, of which the probable error of the mean was calculated to be ± 0.10 milligram. By reference to table 12, it is found that about eighty-one samples are required without taking the laboratory error into account. This error is .009 milligram (table 7) for eighty-one samples, an amount less than one per cent of the error which we are allowing for and hence

negligible, for all practical purposes, when the probable error of the sampling is of as great a magnitude as 0.1 milligram. It must be recognized that the mean of the number of samples so calculated may be found to have a greater or less probable error in practice so that a greater number of samples than calculated should be taken. This relation has already been brought out in the previous section, where it was shown that the fewer the samples, the less representative of the total area they became. By an inspection of table 12, it is evident that the number of samples necessary to secure the degree of accuracy which we established for the residual nitrate samples must be very greatly increased, when we are dealing with ammonium sulfate or blood treated samples.

TABLE 12

PROBABLE ERROR WITH VARYING NUMBERS OF DETERMINATIONS

Number of samples	Residual nitrate		Incubated blanks		Ammonium sulphate		Blood	
	1″–6″	6″–24″	1″–6″	6″–24″	1″–6″	6″–24″	1″–6″	6″–24″
	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.
1	.47	.24	.71	.33	1.01	.74	3.8	3.5
4	.23	.12	.37	.16	.5	.37	1.9	1.7
9	.16	.08	.25	.11	.3	.25	1.2	1.1
16	.12	.06	.18	.08	.25	.19	1.0	0.9
25	.09	.05	.14	.06	.20	.15	.08	0.7
36	.08	.04	.12	.05	.17	.12	0.6	0.6
49	.07	.035	.10	.048	.14	.10	0.5	0.5
64	.06	.03	.09	.04	.12	.09	0.48	0.45
81	.05	.03	.08	.03	.10	.08	0.4	.04

It will be remembered that throughout the discussion, we have considered the probable error of the mean of any given series of samples directly, so that we have but an even chance that the mean for any given number of determinations made will be of significance. It is usual to consider about three times the probable error as a significant difference between two given means, hence if we are to establish this standard as regards the number of samples taken, we must increase all the figures given by three times.* It is obvious that such a number of samples in the case of dried blood, for instance, would be far beyond practical limits as regards the making of the determinations. The chances are that the use of a fewer number of samples means a low degree of reliability for any determinations made. If soil biologists are to continue to make beaker tests with fertilizers, the results must be interpreted from this viewpoint.

* By multiplying the probable error found by 3.17 the chances are thirty to one that a difference greater than the figure so found is significant.

While we are not justified in using the results obtained from laboratory treatments as direct criteria of what will happen in the field, it is probable that greater variability will be found in field plots which have been subject to treatment with fertilizers than in the normal soil to which no fertilizers have been added, especially when the fertilizer is of such a complex nature as dried blood.

It must further be emphasized that the figures as given above apply only to the soil under discussion; each soil with the treatment applied must be considered as a unit in a statistical study. It is evident that a standard established for the residual nitrate, for instance, will not hold for the nitrate produced from a one per cent application of blood. The exact correlation between the various laboratory treatments and between those treatments and results secured in the field is reserved for discussion in a future publication.

Representative and Composite Samples

It is evident from the results already presented in various groupings that a representative sample of any soil is a purely hypothetical quantity whose constituents may only be determined indirectly. It is also evident that determinations reported from only one sample of a given area are practically worthless, when the errors to which it is subject are of the magnitudes shown above. Composite samples made up from a small number of single samples are of little more value. Not until enough samples have been taken to enable the proper calculation of the probable error of the mean of a given number of samples is the making of a composite sample justified, since the results obtained from working with such a sample are of very limited value unless the error to which the composite sample, itself, is subject, can be determined.

It seems, therefore that the use of the word composite as relating to soil samples is only justified when the variations to which the individual samples are subject are known and enough samples taken to establish the error to which the composite, as a mean of all the samples, is liable. The magnitude of the error which is liable to creep into the determinations due to imperfect mixing in a composite sample is no doubt worthy of consideration, but since its value is undetermined, it has been assumed that a composite actually represents the mean of all the samples taken. Just how reasonable such an assumption may be remains for future investigations to bring out.

General Discussion

It has already been stated that the area under discussion was of very limited extent and as free from apparent variations as any area is likely to be. Also, that the treatment and climatic influences had all tended toward a state of biologic equilibrium as regards nitrate production in this particular soil. With this viewpoint in mind, the importance of applying a statistical interpretation to results obtained from working with a given soil type obtained under conditions much less favorable, becomes doubly important. It is not at all improbable that variations several times greater than those reported will be found in many instances and the number of samples deemed sufficient in this case must be increased to obtain any considerable degree of accuracy. It is possible that areas more uniform than the one used will be found, but it is hardly probable that such will be the case.

While the present paper deals only with the production of nitrates, not only the variability of the products of microörganic activities, but the chemical constituents of any soil should be studied in a similar manner to determine just how reliable past results in soil investigations may be. It is beyond question that before we can have faith in future results, we must apply the principles outlined. A study of the problems of nitrogen fixation in the soil, which is to be as complete and as carefully controlled as possible, is now under way at this station. Statistical methods are to be applied to the variations in the total nitrogen, total carbon, and nitrates, at least, in two representative soils under observation before a treatment of any kind is applied. In other words, the experimental areas to be used are being standardized as carefully as possible, making due allowance for field variations in the constituents noted above. Further, the field errors due to variations between the samples must not be greater than the experimental error due to the laboratory manipulations, since the increases (or decreases) to be measured are themselves of small magnitude. To be sure, this method of procedure takes much extra time and energy, but it must be employed before the results of any treatments which are made may be correctly interpreted or recommendations based upon the experimental results safely made. It is hoped that some of the results of these preliminary studies may be ready for publication in the not far distant future.

As regards the effect of seasonal variation upon the accuracy of results which may be obtained at one season of the year upon those secured at another period, no data are as yet available which are extensive enough to warrant any conclusion. It is evident that if a "representative" sample must be found from time to time whenever new work is undertaken, the procedure would become very laborious. The prediction is made that the probable error of the mean as found in any one sampling will be very nearly the same for other samplings taken at other seasons of the year, so that it will only be necessary to make a composite sample of a previously determined number of individual samples and apply the probable error, as previously found, to the results obtained.

The segregation of the causes of biologic variation in the determinations as made, is of importance. Some evidence has already been presented showing the increased variability due to laboratory treatment. Variations in moisture present under incubator conditions, the change in the medium due to the addition of various fertilizers, and the mixing of the fertilizer with the soil are all causal factors in the variations between samples as regards the amount of ammonia, nitrate, or similar compounds finally measured. We are attempting, in this laboratory, to determine the extent, and to limit as much as possible, the variations due to manipulation. In the field the only possible cause contributing to increased variability is the method of sampling, and from unpublished results to be reported later the differences between different methods of sampling are of but little moment in a study of field variability. Variations between field samples may only be allowed for as already outlined and are no longer serious sources of error when once recognized.

There can be no doubt that much of the work done in the past, as regards nitrification at least, is open to very serious question in the light of the results herein reported. Just how much of it can be retained can only be determined by a careful checking of the actual determinations made, taking into account the error due to sampling and to the laboratory manipulations. If the data reported in table 4, for instance, be arranged in any proper order, it will be found that nearly any desired series of results may be secured, agreeing closely in their relative range with any series of results obtained from plots or tumbler treatments which have been reported by various investigators. Individual investigations are not referred to here in detail, since their large number precludes their discussion in the limited space

of this paper. It is beyond question that the soil is an extremely variable quantity and that its variability must be measured and taken into account before any determinations made upon it become of value.

Summary

A study of variability as regards nitrate production of eighty-one samples of soil taken from a limited area of an apparently uniform soil, is reported. Both surface and subsoil samples were taken. The nitrate present at the time of sampling, the nitrate produced from the soil nitrogen, from ammonium sulphate, and from dried blood were determined. Statistical methods were applied for the interpretations of the results. The following conclusions seem justified from the data presented:

1. The variability of the field samples of soil, even from an apparently uniform area of limited extent, is high and is a factor of extreme importance in an estimation of the reliability of any series of results.

2. The variability of the samples treated as in the tumbler method for nitrification studies is increased over that found for the nitrate produced in the field.

3. Subsoil samples vary more in the field, and when treated with fertilizers in the laboratory, than surface samples taken from the same area. No explanation of this fact can be offered at the present time.

4. A single sample of any soil is of little value as regards determinations which may be made upon it.

5. A limited number of samples as ten or sixteen, are subject to wide variations and can only be used when the results are to be interpreted as having a low degree of accuracy.

6. A composite sample may be considered as of value only after the probable error to which it is subject is known and this can only be determined by the use of a large number of individual samples.

7. The distance apart samples are taken is of little importance as long as the samples are uniformly distributed over an area which is apparently uniform.

8. In the light of these results, the conclusion seems inevitable that much of the past work done, as regards nitrification at least, must be critically examined to determine the degree of reliability, if any, it may have.

Attention is called to the fact that variations in other products of microörganic activity and in the chemical constituents of different samples of the same soil may be as large or even larger than those found in the present study; and that a much more comprehensive study of variability than the one herein reported is now being carried out.

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